THE INFLUENCE OF ACTH AND CORTISONE UPON EXPERIMENTAL ACHORION QUINCKEANUM INFECTION AND UPON ANAPHYLAXIS IN GUINEA PIGS*

FREDERICK REISS, M.D. AND LEONA CAROLINE, A.B.

Experimental and clinical data which give evidence of the influence of cortisone and ACTH on allergic and immunologic phenomena are numerous (1–9). Diminution as well as increase of tuberculin sensitivity has been observed during and shortly after cortisone medication, not only in experimental animals (10) but also in man (11, 12). The present study deals with the effect of cortisone and ACTH on the course of *Achorion Quinckeanum* infection in guinea pigs as well as the influence of *long-continued* administration of these drugs upon anaphylactic shock.

METHODS USED IN THE STUDY OF THE COURSE OF *A. QUINCKEANUM* INFECTION

Twelve male guinea pigs (Rockland Farms), weighing 350 gms. each, were maintained on Rockland guinea pig ration, which is fortified with ascorbic acid, and were infected on a shaved and scarified area of the flank with *A. Quinckeanum* ✻ RV 683†. In the guinea pig this infection follows a regular pattern, (see for example reference 13). In such a model infection not only alterations in the course of the infection can be observed with accuracy but changes in cutaneous allergic reactions can be noted.

Four animals served as controls, four guinea pigs received 2.0 mgm. cortisone (cortisone acetate in suspension, Merck‡) twice daily by the intramuscular route in the thigh. Of these animals, two received the cortisone for 10 days prior to the infecting inoculation and in two guinea pigs treatment was initiated simultaneously with the inoculation.

Four additional animals were given ACTH (Adrenocorticotropin, Armour§) 2.0 mgm. twice a day, dissolved in sterile distilled water immediately prior to intramuscular injection. Two of these animals received the hormone for 10 days prior to the inoculation and two animals received the hormone from the date of inoculation. Injections were continued for between 64–71 days throughout the entire course of both the *infection* and the *reinfection* until the final clearing. The onset and duration of the infection were not only evaluated by the appearance of typical scutula but also by the presence of fungi in the scrapings and by positive culture.

* From the Department of Dermatology and Syphilology of the New York University Post-Graduate Medical School (Dr. Marion B. Sulzberger, Chairman) and the Service of Dermatology and Syphilology of Bellevue Hospital (Dr. Frank C. Combes, Chief of Service).

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§ Most of the ACTH used in this study was kindly supplied by Armour and Company.

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Reinoculation with *A. Quinckeanum* on the opposite flank was instituted after the disappearance of the lesions and negative culture findings. Trichophytin tests were done from 3–7 times at intervals of from 12 to 60 days after the first infection, using Trichophytin, Lederle diluted 1:15, 1:30 and also undiluted; and once with Trichophytin Hoechst undiluted and Arlington Allergenic Extracts of Trichophyton Mixture as well as Achorion Schoenleini used undiluted. Readings of the tests were made in 48 hours.

RESULTS

Effect of cortisone on the course of infection

In the cortisone treated animals the *incubation* time of the primary infection was not shortened. However the *course* of the infection in the *cortisone-treated animals showed an increased duration* of the infection, the average time required for clinical healing *being 16 days longer* than in the untreated control animals. The difference between the animals receiving cortisone before inoculation and those receiving it with the inoculation and during the course of the disease was not appreciable. The time for development of the second infection (reaction time of reinfection) was neither shortened nor lengthened in the cortisone treated animals; and the course of the reinfection did not show a significant difference as compared with the controls. There was no marked difference in the severity or extent of the lesions in the two groups. (See Table I)

Effect of ACTH on course of infection

When compared with the controls, the ACTH-treated group showed no differences in the incubation time or the course of the first infection, nor in time of development (reaction time) of reinfection, nor in the course of the reinfection. Like in the cortisone-treated animals, there was no discernible difference in the severity of the infection.

Trichophytin Tests: Despite the fact that a clinical and mycologically proven infection was produced in all these animals, the trichophytin reactions were variable and irregular in all three groups, (controls, cortisone-treated and ACTH-treated), even though we used several different preparations. However positive trichophytin reactions were observed in some of the cortisone- and ACTH-treated animals as well as in some of the controls. The Arlington preparations, when used undiluted, caused a necrotic reaction which was observed even in control animals never infected with *A. Quinckeanum*. These necrotic reactions appearing in all the animals were not considered an allergic phenomenon, but due to some primary necrotizing constituent (perhaps the cresol content of the preparation?) (N.B. for human use these preparations are diluted 1:100; for guinea pig testing we used undiluted material).

General Condition: The general condition of the animals remained good throughout the experiments. Only two animals showed untoward reactions. One developed a paralysis of the left hind leg which disappeared a week after discontinuation of the cortisone therapy and did not reappear when therapy was started

again. One of the ACTH-treated group developed a diarrhea which persisted for two weeks after therapy was discontinued. Another cortisone-treated animal developed a pustular infection in one spot behind the ear which cleared up with the application of ammoniated mercury ointment.

TABLE I

*Incubation period and duration of infection in 12 male guinea pigs, infected with A. Quinckeanum and treated with ACTH or cortisone and in untreated controls**

GROUP	COLOR	GUINEA PIG #	FIRST INFECTION		REINFECTION		NOTES
			Incubation period days	Time to heal days	Incubation period or reaction time days	Time to heal days	
Controls (untreated)	Blackish brown	23	5	19	4	16	
		5	5	25	2	12	
	White	18	6	20	1	13	
	White	18	6	20	1	13	
	White	25	6	21	1	18	
Average				21.2		14.7	
ACTH treated	White	9	5	24	2	20	Therapy simultaneously
	Black	3	5	25	2–3	12	Therapy simultaneously
	Tan	14	5	32	1	14	Therapy prior to infection
	White	2	5	26	2–3	12	Therapy prior to infection
Average				26.7		14.5	
Cortisone treated	White	12	6	51	1	11	Therapy simultaneously
	Tan	16	6	28	1	12	Therapy simultaneously
	Tan	19	6	38	1	14	Therapy prior to infection
	White	20	5	32	1	12	Therapy prior to infection
Average				37.2		12.2	

* The above figures were derived by scoring data from the daily records of the animal without knowing the treatment given to the individual animals.

Anaphylactic Shock

The mechanism of the effect of ACTH and cortisone on various allergic and immunologic phenomena is still unknown. Several investigators (13, 14, 15) have reported observations on both active and passively induced anaphylactic shock in guinea pigs which had received cortisone or ACTH either simultaneously with the shocking injection or for a period of 7–10 days prior to the shocking dose, None of these authors had utilized animals which had been treated over a longer period with ACTH or cortisone. Since we had at our disposal several animals which had been treated with *ACTH or cortisone for 7 months* it was of particular

interest to investigate the effect of such long-continued hormonal administrations on the classic anaphylactic reaction.

METHODS AND RESULTS IN THE ELICITING OF ANAPHYLACTIC SHOCK

Nine male guinea pigs, weighing 600–850 grams, were used and consisted of the following groups: four treated for 7 months with cortisone, 2.0 mgm. twice daily; three treated with ACTH for the same period and the same dosage schedule; and two untreated animals as controls. The animals were sensitized with 0.1 ml. of normal horse serum diluted 1:50 injected subcutaneously*. After three weeks a challenging dose of 1 ml. of normal horse serum diluted 1:10 was injected by the intrajugular route. Treatment with the hormones was continued uninterruptedly to the day of administration of the shocking dose.

TABLE II

Statistical analysis

	DIFFERENCE OF MEAN HEALING TIMES (DAYS)	"STUDENT'S" *t*	P (FOR 9 DEGREES OF FREEDOM)
ACTH minus controls	5.5	1.23	Between 0.3 and 0.2
Cortisone minus controls	16.0	3.57	Less than 0.01
Cortisone minus ACTH	10.5	2.34	Between 0.05 and 0.02

NOTE. The standard error of the difference used here (4.486) was derived from the pooled intragroup variation in the 3-group analysis of variance, and therefore includes the rather marked variation within the cortisone group. When the control and ACTH groups are compared with each other by the standard error of the difference (2.237) derived from them alone, *t* is 2.47, on the verge of significance at the 5 per cent level for 6 degrees of freedom. The result might be called suggestive but not conclusive.

Both controls, all of the cortisone-treated group and one of the ACTH-treated group reacted with fatal anaphylactic shock within 2–13 minutes after the intrajugular injection of the shocking dose, whereas two of the ACTH group survived a mild anaphylactic shock.

STATISTICAL ANALYSIS OF THE DATA ON THE COURSE OF INFECTION†

The three groups (controls, cortisone-treated, ACTH-treated) did not differ significantly in the length of the first incubation period of the fungous infection. Moreover the three groups produced the usual shortened reaction time for development of the reinfection with the same strain of fungus. (Accelerated response.)

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The differences in days to healing (first infection minus second infection) were tested by analysis of variance, the 5% point (1 chance in 20) being taken as the criterion of significance. The mean of the differences in the cortisone-treated animals was significantly greater than in the controls and the ACTH-treated animals; but controls and the ACTH-treated animals did not differ significantly from each other.

There was no significant difference between the effect of prior and simultaneous administration of the hormones. Furthermore there was no appreciable difference in animals of different color.

The interpretation of the significant effect of cortisone on healing time was found by further analysis. The three groups did not differ significantly in the average time of healing after the second infection. In the first infection, however, there was a significant difference among the three groups, and following comparisons show where the chief difference lay.

DISCUSSION

Our studies indicate that ACTH has no demonstrable effect upon the course of *A. Quinckeanum* infection in the guinea pig. Cortisone, however, extended the duration of the first infection but did not interfere with the course of the reinfection. No effect was observed on the trichophytin reaction. Similar studies were made by Kligman and coworkers (16) investigating the effect of 5 mgm. of cortisone per day on *Trichophytin gypseum* infection in the guinea pig, and by Werner Jadassohn et al. (17) on the effects of doses up to 15 mgm. of cortisone per day upon the course of *A. Quinckeanum* infection. The latter authors did not observe any appreciable difference in the incubation or evolution of the first infection or of the reinfection. However Kligman, whose dosage schedule more closely resembled ours, also observed a prolongation of the first infection in the cortisone treated guinea pigs; but he observed cortisone-produced changes in the course of the *reinfection*, which were not observed by either Jadassohn and coworkers (17) or by us.

We are unable at present to offer any exact serological or biochemical evidence regarding the mechanism of the activity of cortisone in prolonging the course of the first infection in Kligman's series and ours. The decrease in macrophage activity, the depletion of lymphocytes and the reduced hyalouronic acid content (all of which occur during cortisone therapy) are not yet proved as causes of the prolongation of this type of local infection.

As far as regards the classic anaphylactic experiment, despite the fact that our animals received ACTH or cortisone for 7 months, neither cortisone or ACTH prevented the production of anaphylactic shock, although two out of three guinea pigs treated with ACTH experienced only mild shock reaction and survived. These experiments are in agreement with Landau (14), Malkiel (15) and Friedlander (13) who were unable to protect guinea pigs against histamine shock or against anaphylactic shock studied in the actively and passively sensitized guinea pigs.

Under the conditions of our experiments there is no evidence that cortisone

and ACTH medication so depress antibody formation as to prevent the development of trichophytin sensitivity or anaphylactic sensitivity.

SUMMARY

Among twelve guinea pigs whose skins were infected with *A. Quinckeanum*, four were treated with ACTH and four with cortisone. While ACTH did not change the course of the disease, the cortisone treated animals showed a significant prolongation of the first infection; but no change in the nature or course of the reinfection with the same strain of fungus. Trichophytin reaction was not altered either by ACTH or Cortisone.

Guinea pigs receiving cortisone daily for seven months and then being actively sensitized with horse serum died in severe anaphylactic shock; while of three guinea pigs, treated with ACTH for 7 months, one died in shock and two survived a mild but typical anaphylactic reaction.

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